UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

January 21, 2003

MEMORANDUM

Subject: Efficacy Review for EPA Reg. No. 9150-2, 9150-3
DP Barcode: D284898, D284897

From: Ian Blackwell, Biologist
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Applicant: International Dioxcide

Formulation From Label:

Active Ingredient(s)	% by wt	
	9150-2	9150-3
Chlorine Dioxide	2	5
Inert Ingredient(s)	95	98
Total	100	100

I **BACKGROUND:** International Dioxcide has submitted two antimicrobial efficacy studies, and, an addendum to one of the two studies. These studies were submitted in support of their products, "Anthium Dioxcide 5% Aqueous Stabilized Chlorine Dioxide" and "Carnebon 200 2% Aqueous Stabilized Chlorine Dioxide".

Anthium Dioxcide (EPA Reg. No. 9150-2), is an EPA-approved food-contact surface sanitizer, disinfectant, deodorizer, slimicide, and mildewstat for use on hard, non-porous surfaces in institutional and industrial settings. Carnebon 200 (EPA Reg. No. 9150-3), is also an EPA-approved food-contact surface sanitizer, disinfectant, deodorizer, slimicide, and mildewstat for use on hard, non-porous surfaces in institutional and industrial settings. The applicant indicates that both products contain the same ingredients and that Anthium Dioxcide (5% chlorine dioxide) is a more concentrated version of Carnebon 200 (2% chlorine dioxide). The applicant requested to make the same revisions to both product labels. With regard to product efficacy claims, the applicant requested to:

- revise the disinfectant instructions to reflect a less concentrated use solution (i.e., 300 ppm total available chlorine dioxide when activated to a pH of 2.6);

- revise the food-contact surface sanitizer instructions, for the Oxychlor e-generator option, to reflect a less concentrated use solution (i.e., 50-100 ppm total available chlorine dioxide);

- add a "me-too" use for the "treatment of water and ice associated with fish in the round";

- add a new claim of effectiveness against Foot and Mouth Disease;

- revise the cleaning instructions for highly-contaminated recirculating cooling towers to reflect a more concentrated use solution (i.e., from 5 to 20 ppm total available chlorine dioxide).

The studies were conducted at MicroChem Laboratory, Inc. located at 7423 Airport Freeway in Fort Worth, Texas 76118; International Dioxcide, Inc. located at 554 Ten Rod Road in North Kingston, Rhode Island 02852; and, the Foreign Animal Disease Diagnostic Laboratory at the United States Department of Agriculture (USDA).

This data package contained EPA Form 8570-1 (Application for Pesticide) for both products, three studies (MRID Nos. 457267-01, 457267-02, 457209-01 and 457209-02), Statements of No Data Confidentiality Claims for all four studies, the proposed label for Anthium Dioxcide (date stamped by EPA on July 17, 2002), and the proposed label for Carnebon 200 (date stamped by EPA on July 17, 2002). MRID Number 457209-01 is simply an addendum to 457267-02.

These studies were primarily reviewed by the EPA contractor DynCorp Systems & Solutions LLC. A secondary review was conducted by EET/PSB/AD scientists.

II Use Directions

The proposed labels for both products provided the following directions regarding preparation and use:

Use as a disinfectant:

For 9150-2: Clean surfaces with a suitable detergent. Rinse with water. Prepare a use solution containing 300 ppm available chlorine dioxide by adding, for example, 0.8 fluid ounces of product to 1 gallon of water, followed by pH adjustment to 2.6 with acetic, citric, phosphoric, sulfuric, or hydrochloric acid. Thoroughly wet surfaces with the use solution using a sprayer, mop, or sponge. Allow surfaces to remain wet for 10 minutes. Air dry.

For 9150-3: Add 2 fl. oz of Carnebon 200 to one gallon of water into a clean plastic pail and add 1.2 grams of Activator-C. This will yield a working solution containing 300 ppm of available chlorine dioxide. Allow 15 minutes reaction time and for activator to dissolve completely.

Use as a sanitizing rinse on previously cleaned food-contact surfaces (Oxychlor e-generator option): Remove gross food particles and soil. Clean surfaces with a detergent or cleaner. Rinse with potable water. Prepare a use solution containing 50-100 ppm available chlorine dioxide using the Oxychlor e-generator. Apply the use solution to surfaces making sure that the solution contacts all surfaces for at least 1 minute. Allow surfaces to drain. Air dry. Do not rinse treated surfaces.

Use as a disinfectant against Foot and Mouth Disease virus: Clean surfaces with a suitable detergent. Rinse with water. Prepare a use solution containing 390 ppm available chlorine dioxide by adding 1 fluid ounce of product to 1 gallon of water, followed by pH adjustment to 2.6 with acetic, citric, phosphoric, sulfuric, or hydrochloric acid. Thoroughly wet surfaces with the use solution using a sprayer, mop, or sponge. Allow surfaces to remain wet for 20 minutes. Air dry. Before re-use, thoroughly scrub feeders and waterers with detergent and rinse with potable water. Note: For the product, Carnebon 200, prepare a use solution containing 390 ppm available chlorine dioxide by adding 2.5 fluid ounces of product to 1 gallon of water, followed by pH adjustment to 2.6.

Use as a bacteriostat for treating water and ice associated with fish in the round: Prepare a use solution containing 20 ppm total available chlorine dioxide. Apply use solution to water and ice that are used to rinse, wash, thaw, transport, or store seafood. Rinse with potable water any seafood that is intended to be consumed.

III Agency Standards for Proposed Claims

Disinfectants for Use on Hard Surfaces in Hospital or Medical Environments

The effectiveness of disinfectants for use on hard surfaces in hospital or medical environments must be substantiated by data derived using the AOAC Use-Dilution Method (for water soluble powders and liquid products) or the AOAC Germicidal Spray Products Test (for spray products). Sixty carriers must be tested with each of 3 product samples, representing 3 different batches, one of which is at least 60 days old, against *Salmonella choleraesuis* (ATCC 10708), *Staphylococcus aureus* (ATCC 6538), and *Pseudomonas aeruginosa* (ATCC 15442). To support products labeled as "disinfectants", killing on 59 out of 60 carriers is required to provide effectiveness at the 95% confidence level.

Sanitizing Rinses (For Previously Cleaned Food Contact Surfaces)

Sanitizing rinses may be formulated with iodophors, mixed halides, or chlorine-bearing chemicals. The effectiveness of such sanitizing rinses for previously cleaned food contact surfaces must be substantiated by data derived from the AOAC Available Chlorine Germicidal Equivalent Concentration Method. Data from one test on each of 3 product samples, representing 3 different batches, one of which is at least 60 days old against *Salmonella typhi* (ATCC 6539) are required. Test results must show product concentrations equivalent in activity to 50, 100, and 200 ppm of available chlorine. The reference standard is sodium hypochlorite.

Virucides

The effectiveness of virucides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. Carrier methods that are modifications of either the AOAC Use-Dilution Method (for liquid disinfectants) or the AOAC Germicidal Spray Products Test (for spray disinfectants) must be used in developing data for virucides intended for use upon dry inanimate, environmental surfaces (e.g., floors, tables, cleaned dried medical instruments). To simulate in-use conditions, the specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the product label. **In the case of Foot and Mouth Disease Virus (FMDV) an exception is made in the requirement for efficacy testing using a carrier based system. FMDV loses viability upon drying, therefore, the use of a suspension test is acceptable.** Two different batches of disinfectant must be tested against a virus titer of at least 10^4 for a specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virological technique. The calculated viral titers must be reported with the test results. For the data to be considered acceptable, results must demonstrate complete inactivation of the virus at all

dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level.

IV Comments on the Submitted Efficacy Studies

1 MRID 457267-01: "A Study of the Bactericidal Activity of 300 ppm Chlorine Dioxide, Anthium Dioxcide Disinfectant by the Methods of the AOAC Use Dilution Test with Ten Minutes Exposure at 20±1°C", by Michelle Taylor. Study conducted at MicroChem Laboratory, Inc. Lab Project ID Numbers – 991124-1, 991206-1 and 991207-1. Study completion date – December 21, 1999.

This study was conducted against *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 15442) and *Salmonella choleraesuis* (ATCC 10708) in the presence of a 5% organic soil load (heat-inactivated calf serum). Three lots (Lot Nos. 651, 662, and 666) of the product, Anthium Dioxcide, were tested using the AOAC Use-Dilution Method as described in the AOAC Official Methods of Analysis, 15th Edition, 1990. One product lot (Lot No. 651) was at least 60 days old at the time of testing. Sixty (60) carriers were tested per lot of disinfectant against each test organism. A use solution containing 300 ppm total available chlorine dioxide was prepared. The use solution was not prepared using the Oxychlor e-generator. Sixty stainless steel cylinders were soaked (20 at a time) in 20 mL of bacterial culture and allowed to dry for 40 minutes at 35±2°C. Each contaminated carrier was placed in 10 mL of the use solution at 30 second intervals for an exposure time of 10 minutes at 20±1°C. After exposure, the carriers were transferred to tubes of 10 mL of Dey-Engley Neutralizing Broth, incubated for 48±8 hours at 35±2°C, and observed for growth or no growth. Controls included viability, numbers control, phenol resistance, neutralization validation, and sterility.

2 MRID 457267-02: "A Study of Anthium Dioxcide Disinfectant, a Chlorine Dioxide Solution, to Kill Bacteria in a Manner Equivalent to Known Concentrations of Available Chlorine", by Sara Stumph. Study conducted at MicroChem Laboratory, Inc. Lab Project Number – 010921-1. Study completion date – October 4, 2001.

This study was conducted against *Salmonella typhi* (ATCC 6539) in the presence of a 5% organic soil load (heat-inactivated calf serum). Three lots (Lot Nos. SD-714, SD-719, and SD-721) of the product, Anthium Dioxcide, were tested using the AOAC Chlorine (Available) in Disinfectants Germicidal Equivalency Test as described in the AOAC Official Methods of Analysis, 16th Edition, 1995. One product lot (Lot No. SD-714) was at least 60 days old at the time of testing. Use solutions containing 50 ppm total available chlorine dioxide were prepared for all three lots. The manufacturing dates for the three product lots were provided in MRID No. 457209-01. A use solution containing 100 ppm

total available chlorine dioxide was prepared for one lot (Lot No. SD-714). NaOCl control solutions of 200, 100, and 50 ppm available chlorine were prepared. The use solutions were prepared electrolytically by adding the product, Anthium Dioxcide, directly to the Oxychlor e-generator. MRID No. 457209-01 provides additional information about how the use solutions were prepared. A 0.05 mL aliquot of the test suspension was added to 10 mL of each of the use solutions and NaOCl control solutions at 20±1°C. After one minute, a loopful (i.e., 0.05 mL) of each culture-solution mixture was transferred into 10 mL of Dey Engley Neutralizing Recovery Medium. Each tube was then challenged with an additional 0.05 mL aliquot of the test suspension 30 seconds after subculturing. This procedure was repeated for a total of 10 subcultures for each use solution and NaOCl control solution. All tubes were incubated at 35±2°C for ≥48 hours and observed for growth or no growth. Controls included viability, numbers control, phenol resistance, neutralization validation, and sterility. Total chlorine dioxide was measured by iodine titration analysis. Free chlorine dioxide was measured by light absorption at 360 nm plus the constant factor.

3 MRID 457209-02: "Efficacy of Anthium Dioxcide Against Selected Foreign Animal Disease Agents" by Carol House. Study conducted at Animal and Plant Health Inspection Service (APHIS) Foreign Animal Disease Diagnostic Laboratory at the United States Department of Agriculture (USDA). Study completion date - July 14, 1994.

Testing was conducted against Foot-and-mouth disease virus, serotype O_1 Campos. The cell line for determining infectivity was IBRS-2 (**I**nstituto **B**iologico **R**im **S**uino pig kidney 2)100 µl/well at 4 x 10^5 cells/mL. Dilute Anthium Dioxide 1:140 in water, adjust pH to 3.0 with citric acid. Allow to set at room temperature for 15 minutes prior to use, to yield a final concentration of 390 ppm chlorine dioxide. Equal volumes (0.75 mL of each) of virus (in EMEM, supplemented with 2.5% FCS) and 2x disinfectant solution were mixed and incubated for 5 minutes at 25°C. A virus control, using supplemented EMEM instead of disinfectant and a toxicity control, using media instead of virus were set up in the same manner as the test disinfectant/virus mixture. At the end of the incubation period, each mixture is poured onto a prepared column (Sephadex LH- 50) and centrifuged 15 minutes at 1800 rpm (1000 x g). The fluid is collected from each column and titrated in microtiter plates using 10-fold dilutions in supplemented EMEM solution, against IBRS-2 cells. The plates are incubated at 37°C, 95% humidity, 5% CO_2 for 3 days and then read for cytopathic effects (cpe). Three sets of replicates are performed on different days. The titer is calculated as $TCID_{50}$/25 µl.

V Results

Anthium Dioxcide at 300 ppm Chlorine Dioxide					
MRID Number	Organism	No +/Total No. Tested			Viability (CFU/carrier)
		Lot No. 651	Lot No. 662	Lot No. 666	
457267-01	*Staphylococcus aureus*	0/60	0/60	0/60	3.70×10^5
	Pseudomonas aeruginosa	0/60	0/60	0/60	4.01×10^6
	Salmonella choleraesuis	0/60	0/60	1/60	3.68×10^6

MRID Number	Organism	Lot or Concentration	Subculture Series									
			1	2	3	4	5	6	7	8	9	10
457267-02	*Salmonella typhi*	50 ppm										
		Lot No. SD-714	0	0	0	0	0	+	+	+	+	+
		Lot No. SD-719	0	0	0	0	0	+	+	+	+	+
		Lot No. SD-721	0	0	0	0	+	+	+	+	+	+
		100 ppm										
		Lot No. SD-714	0	0	0	0	0	0	0	+	+	+
456267-02	*Salmonella typhi*	NaOCl 200 ppm	0	0	0	0	0	0	+	+	+	+
		NaOCl 100 ppm	0	0	0	+	+	+	+	+	+	+
		NaOCl 50 ppm	0	0	+	+	+	+	+	+	+	+

Note: The initial suspension population was 3.52×10^8 CFU/mL.
+ = Growth of organism
0 = No growth of organism

MRID # 457209-02: Complete inactivation of virus in all three replicates.

VI Conclusions

The conclusions reported in this section of the report are based on studies that used Anthium Dioxcide as the test substance. In all cases, the conclusions are equally relevant to the product, Carnebon 200, as the two products – Anthium Dioxcide and Carnebon 200 – reportedly differ only in the concentration of the active ingredient, chlorine dioxide and are to be applied at the same use concentrations.

1 Anthium Dioxcide

A MRID No. 457267-01: The submitted efficacy data support the use of the product, Anthium Dioxcide, as a disinfectant when tested against *Salmonella choleraesuis* (ATCC 10708), *Staphylococcus aureus* (ATCC 6538), and *Pseudomonas aeruginosa* (ATCC 15442) in the presence of a 5% organic soil load (heat-inactivated calf serum) on hard, non-porous, inanimate surfaces for a contact time of 10 minutes at the label-specified dilution (i.e., 300 ppm available chlorine dioxide; prepared when the product is activated to a pH of 2.6). No growth was observed in the subcultures of the 60 carriers tested against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Growth was observed in the subculture of only 1 of 60 carriers tested against *Salmonella choleraesuis* (i.e., Lot No. 666). The dried carrier counts were at least 10^4 CFU/carrier. The recovery medium was sterile and supported the growth of low numbers of bacteria. Neutralization validation testing showed positive growth of the test organisms.

B MRID Nos. 457267-02 and 457209-01: As tested against *Salmonella typhi* (ATCC 6539) with a contact time of 1 minute at the label-specified dilution (i.e., 50-100 ppm available chlorine dioxide; prepared using the Oxychlor e-generator), the submitted efficacy data support the use of the product, Anthium Dioxcide, as a sanitizing rinse on previously cleaned, hard, non-porous, inanimate food-contact surfaces. Test results of the product, Anthium Dioxcide, when diluted at 50 ppm available chlorine dioxide, show the absence of growth in at least 4 consecutive tubes of subculture. The NaOCl control solution (at 50 ppm available chlorine) shows the absence of growth on only 2 consecutive subcultures. Therefore, when compared to the NaOCl control solution (at 50 ppm available chlorine), the product (diluted at 50 ppm available chlorine dioxide) was observed to be as, or more, effective than the NaOCl control solution. The product (diluted at 50 ppm available chlorine dioxide) was also observed to be as, or more, effective than the 100 ppm available chlorine NaOCl control solution. The *Salmonella typhi* culture in 0.05 mL was determined to contain 1.76×10^7 CFU/mL. Neutralization validation testing showed positive growth of the test organism. Phenol resistance testing was reported. Although the applicant reported that sterility testing was performed, the results for this test were not reported.

Note: Only one product lot was tested at a dilution of 100 ppm available chlorine dioxide (i.e., Lot No. SD-714). This product solution, when compared to each of the three NaOCl control solutions, was observed to be as, or more, effective than the NaOCl control solutions.

C MRID 457209-02: In the three trials conducted with Anthium Dioxide, the product demonstrated 100% inactivation of the foot-and-mouth disease virus

when tested at 390 ppm chlorine dioxide for 5 minutes at 25°C. Cytotoxicity was not detected in any of the tested samples. However, this data does not support the addition of a claim against FMDV on the label because only one batch was tested and the testing was not conducted under the Good Laboratory Practice Standards.

2 Carnebon 200

A MRID No. 457267-01: The submitted efficacy data also support the use of the product, Carnebon 200, as a disinfectant when tested against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella choleraesuis* in the presence of a 5% organic soil load (heat-inactivated calf serum) on hard, non-porous surfaces for a contact time of 10 minutes at the label-specified dilution (i.e., 300 ppm available chlorine dioxide; prepared when the product is activated to a pH of 2.6).

B MRID Nos. 457267-02 and 457209-01: The submitted efficacy data also support the use of the product, Carnebon 200, as a sanitizing rinse on previously cleaned food-contact surfaces when tested against *Salmonella typhi* on hard, non-porous surfaces for a contact time of 1 minute at the label-specified dilution (i.e., 50-100 ppm available chlorine dioxide; prepared using the Oxychlor e-generator).

C MRID No. 457209-02: The submitted efficacy data does not support the use of Carnebon 200 as a virucide against FMDV because only one batch was tested and the testing was not conducted under the Good Laboratory Practice Standards.

VII Recommendations

Anthium Dioxcide and Carnebon 200

1 The proposed label claims (as supported by MRID No. 457267-01) are acceptable regarding the use of the product, Anthium Dioxcide/Carnebon 200, as a disinfectant on hard, non-porous surfaces for a contact time of 10 minutes at the label-specified dilution (i.e., 300 ppm available chlorine dioxide; prepared when the products are activated to a pH of 2.6).

2 The proposed label claims for disinfecting "commercial animal confinement facilities such as poultry houses, swine pens, calf barns and kennels" when use solutions are prepared using the Oxychlor e-generator are denied. The Agency requests that the applicant submit specific data for disinfection using the Oxychlor e-generator.

3 The proposed label claims (as supported by MRID Nos. 457267-02 and 457209-01) are acceptable regarding the use of the products, Anthium Dioxcide and Carnebon 200, as a sanitizing rinse for previously cleaned food-contact surfaces on hard, non-porous surfaces for a contact time of 1 minute using a dilution yielding 50-100 ppm available chlorine dioxide. This claim applies only to use solutions prepared using the Oxychlor e-generator.

4 The proposed label claim for Anthium Dioxcide and Carnebon 200 (MRID 457209-02) as a virucide against the Foot and Mouth Disease Virus is denied. The submitted data could be upgraded for registration if another batch of the product was tested, under the same conditions as the batch in MRID 457209-02,, or evidence was submitted that the testing conducted in 1994, was done under Good Laboratory Practices (GLP) against 2 separate batches of the product, and demonstrated complete inactivation of FMDV.

5 The proposed label claims [see page 11 of the label] for the products, Anthium Dioxcide and Carnebon 200, regarding use as a bacteriostat for treating water and ice associated with fish in the round are denied. The applicant states that this claim has been accepted for the product, Oxine (EPA Reg. No. 9804-1), which has 2% chlorine dioxide as the active ingredient. However, the use directions proposed for Oxine and Anthium (Carnebon 200) are not the same.

6 The proposed label claims [see page 9 of the proposed label] for the products, Anthium Dioxcide and Carnebon 200, are acceptable regarding the use of a solution containing 5-20 ppm available chlorine dioxide to control slime growth in heavily contaminated recirculating cooling towers. These claims for the control of microorganisms of economic or aesthetic significance are not directly related to human health and, thus, do not require the submission of efficacy data. The proposed labels appear to provide adequate dosage recommendations and complete directions for use.

7 The proposed label for the products, Anthium Dioxcide and Carnebon 200, includes a claim that was not listed on the last accepted label. The proposed label includes directions for use "to disinfect commercial animal confinement facilities such as swine pens and barns against . . . Swine Vesicular Disease Virus, and African Swine Fever Virus." Although these two organisms are not human health related, due to their socio-economic impact, the Agency is requiring the submission of efficacy data to support the label claims. Data should be developed using a carrier based test unless there is documented evidence that the viruses lose viability when dried on carriers. As of this writing, no claims for 9150-2 or 9150-3 against Swine Vesicular Disease Virus, or African Swine Fever Virus have been approved. These label claims must be removed until they have been substantiated.